ON THE EFFECT OF EXTERNAL CONDITIONS ON THE REPRODUCTION OF DAPHNIA [1]

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SINCE in the great majority of organisms only the germ cells are capable of reproducing the entire individual, the question as to what differences exist between the germ and body cells, and how they arise, is of general interest. Therefore any change of conditions which affects the germ cells or their relation to the body cells deserves special study. In *Daphnia* external conditions not only affect the relation of the germ cells to the body cells, but they affect the egg cells in such a manner as to determine whether they do or do not need fertilization. The purpose of the present paper is not merely to add the results of my experiments to those of other investigators, but to tentatively arrange the available data under a general working hypothesis in the hope that some more direct method of investigating the relation of the germ and body cells be devised.

Last spring (March 10) I began experiments on the effects of environment on *Daphnia pulex,* De Geer, without knowing that Woltereck was working on the same line. The material came from a small pool investigated by Dr. W. C. Curtis and containing a single strain of this and no other species of *Daphnia.* For the first few weeks some ice remained on the pool and the temperature did not much exceed 4° C.; after this it rose steadily to about 20° by the end of May. Specimens from the pool were examined at intervals as a control on the experiments.[2]

[1] From the Zoological Laboratory of the University of Missouri and the Histological Laboratory of Cornell University Medical College, New York City.

[2] When the daphnids were crowded in dishes of the same pool water they soon began to die, owing to the accumulation of their excretions. When

Effect of Environment on Differential Growth

By differential growth I mean the unequal growth of different parts, viz., the germ and body cells. Only parthenogenetic females were used, and each was kept separately in the same quantity of water. All measurements were made at sexual maturity, *i. e.*, when the first eggs appeared in the brood pouch. Warren[3] found that under uniform conditions there was a slight variability, but Woltereck showed that these fluctuating variations were very small, though he did find mutations as rare occurrences.

Nutrition.—I had in the laboratory a pure culture of a unicellular green alga which the daphnids ate readily. This alga did not remain entirely suspended in the water, but as the daphnids fed on the bottom as well as while swimming, and stirred up the algæ, it can not be said that most of the food was out of their reach.

Those with a superabundance of food were larger at sexual maturity and had a shorter spine than those with insufficient food, and conversely. The smaller size and longer spine of the starved daphnids are characteristic of immature stages.

Temperature.—Three sets of experiments were

transferred suddenly to artesian tap water many died, though with a gradual change all lived. The composition of the tap water was as follows: Ca, .148 $\times 10^{-8}$ molecular; Mg, .1 $\times 10^{-8}$ molecular; CO_3, .045 $\times 10^{-8}$ molecular; SO_4, .146 $\times 10^{-8}$ molecular; Cl, .055 $\times 10^{-8}$ molecular. Besides these were very small quantities of silica, clay, iron, ammonia and nitrates, and traces of lithium and potassium. Beside the carbonates the water when drawn from the tap was super-saturated with carbon dioxide. In order to find the cause of death from change of water I added various amounts of molecular solutions of certain salts. The toxicity of cations increased as follows: $NA < 1/2\,Ca < K$, and of anions: $Cl < 1/2\,SO_4 < HCO_3 < 1/2\,HPO_4$. But as K became toxic only on 1/100 and HPO_4 on 1/500 molecular concentration there must be other toxic substances than salts in the water. The carbon dioxide in the water killed crayfish and was probably the most toxic constituent to the daphnids.

[3] "An Observation on Inheritance in Parthenogenesis," *Proc. Roy. Soc. London*, 1899, LXV, and "On the Reactions of *Daphnia magna* to certain changes in Environment," *Quart. Jour. Micr. Sc.*, 1900.

started: at 4–10°, 19–20° and 29–31° C. The results were as follows:

	Average Body Length.	Ratio of Average Spine Length to Body Length.	Time from Hatching to Sexual Maturity.
4–10°	22	0.24	35 days
19–21°	20	0.25	14 "
29–31°	17.7	0.27	6 "

1 unit = $\frac{2}{27}$ millimeters.

All were given a surplus of food daily. It may be observed that a higher temperature has the same effect as insufficient food.

Salts.—Since salts have such a marked effect on the development of marine and some fresh-water animals, I placed daphnids in the strongest solutions of various salts that they would live in (without acclimatization). The effects in two months (four generations) were unnoticeable.

Light.—Cultures were kept in the dark and in diffuse and direct sunlight, but no effect was observed.

A number of observers have recorded season-polymorphism in daphnids. Wesenberg-Lund[4] pointed out that when the specific gravity and consequent buoyancy of the water decreased—by heat in summer—the body of the daphnids became smaller or were provided with outgrowths, so as to offer a greater resistance to sinking. Wolfgang Ostwald[5] produced, all at the same time, the forms that occurred in nature at different seasons, by varying the temperature. He emphasized the fact that rise in temperature lowered the internal viscosity of the water. He found that in the warm cultures the daphnids often became productive at an undeveloped stage and, as is true generally, reproduction retarded body growth.

[4] "Ueber das Abhängigkeitsverhältnis zwischen den Bau der Planktonorganismen und den specifischen Gewicht des Süsswassers," *Biol. Centlb.*, 1900, XX, pp. 606–619, 644–656, and "Studier over de Danske söers Plankton," Copenhagen, 1904.

[5] "Experimentelle Untersuchungen über den Saisonpolymorphismus bei Daphniden," *Archiv f. Entwicklungsmech. d. Organismen*, 1904, XVII, p. 415.

Woltereck[6] maintains that Ostwald's results were due to the fact that at a higher temperature the daphnids need more food. Woltereck caused decrease in body length both by starving and by increasing the temperature, but the latter was not effective with an optimum supply of food. He found that more food than the optimum produced effects similar to starving. Raising the internal viscosity of the water by adding quince gum produced no effect. He showed that though feeding influenced the differential growth, there was a cyclical tendency for this to vary, viz., season-polymorphism. However, the effects of prolonged abundant feeding were inherited to some degree.

One might interpret these results in different ways. It is known that the temperature coefficients for various chemical reactions are slightly different. Possibly the mean of the temperature coefficients for the processes in the development of the reproductive organs is higher than the same for the body wall, and at a higher temperature the germ cells would develop faster. However, under adverse conditions the "affinity" of the reproductive organs for nutriment is greater than that of the rest of the body, so with deficient food the body wall is retarded more than the germ cells in development. The higher temperature may be considered an adverse condition since the mortality is greater. In this way starving has the same effect as a higher temperature.

Langerhans[7] found that accumulation of excretions caused shortening of the spine in daphnids. I do not know what relation this bears to the above results.

[6] "Ueber naturliche und kunstliche Varietätenbildung bei Daphniden," *Verh. Deutsch. Zool. Gesell.*, 1908, p. 234; and "Weitere experimentelle Untersuchungen über Artveranderung, speziell über das Wesen quantitativer Artunterschiede bei Daphniden," *ibid.*, 1909, p. 110.

[7] "Ueber experimentelle Untersuchungen zu Fragen der Fortpflanzung, Variation und Vererbung bei Daphniden," *Verh. Deutsch. Zool. Gesell.*, 1909, p. 281.

Effect of Environment on the Life Cycle

In most species of daphnids, generations of parthenogenetic females alternate with generations of males and females which produce eggs that must be fertilized, and either frozen, dried or kept a long time before they will develop (resting or "winter" eggs). In different species the number of successive parthenogenetic generations varies. In some all are, and in some none are, parthenogenetic.

I found that heat hastened the appearance of sexual forms, as did starving or the accumulation of excretory products. All of these factors might be combined in the drying up of a pond, as heat would aid in drying, and drying would concentrate the daphnids and their excretions, and concentration of the daphnids would cause them to eat up the algæ faster than they could multiply. However, by keeping the culture cold, fresh or well-fed, or all combined, I could delay but not prevent the appearance of sexual forms.

Kurz[8] said the drying up of the water caused the appearance of sexual forms, and Schmankewitz[9] suggested that it was the increase in salts. Weismann[10] tested both of these hypotheses and concluded that they were wrong. He also tried the effect of food and temperature, with varying results. He concluded that the life cycle was fixed for each species and variety. Issakowitz[11] concluded that cold favored the appearance of sexual forms and warmth favored the parthenogenetic. Also, hunger favored the appearance of sexual forms and abundant food the parthenogenetic. It may be that cold retarded multiplication of the food plant or the

[8] "Dodekas neuer Cladoceren nebst einer kurzen übersicht der Cladocerenfauna Böhmens," *Sitz. Ber. math. naturw. Wien,* 1875.

[9] "Zur Kenntnis des Einflusses der ausseren Lebensbedingungen auf die Organisation der Tiere," *Zeit. wiss. Zool.,* 1877, XXIX.

[10] "Beiträge zur Naturgeschichte der Daphnoiden, VII," *Zeits. wiss. Zool.,* XXXIII, p. 111.

[11] "Geschlechtbestimmende Ursachen bei den Daphniden," *Biol. Centralb.,* 1909, XXV, pp. 529–536.

movements of the daphnids so that they did not keep the algæ stirred up in the water sufficiently to get at them. The parthenogenetic egg arises from four cells, but a large number of cells enter into the composition of the fertilizable egg. If the latter egg is not fertilized it is absorbed and, as Issakowitsch noted, furnishes food for the development of parthenogenetic eggs.

Woltereck[12] found that starving hastened the appearance of sexual reproduction, but a concentration of food above the optimum produced results similar to starving. He found, as Weismann maintained, a cyclical tendency toward the alternation of sexual and parthenogenetic generations which, contrary to Weismann, was temporarily influenced by nutrition, and the effects of constant nutrition over a long period was inherited to some extent.

Langerhans[13] found that the accumulation of excretions caused the decrease in numbers of parthenogenetic females in the autumn and thinks that the appearance of sexual forms is due to the same cause.

The life cycle of a daphnid is, therefore, an hereditary tendency, but can be influenced by nutrition and probably by temperature and the accumulation of excretions. Nutrition is the most important factor, and former experiments on the effect of temperature and the drying up of the water were complicated by secondary effects on concentration of food and excretory products.

Discussion of Results.—Two views might be held as to the origin of the differences between the germ and body cells: the differences might be the result of difference in position in the embryo, or of unequal mitoses. In the parasitic copepods I found the primary germ cell to arise by an unequal mitosis at the fifth cleavage of the egg. The germ cell when first formed is one thirty-second of the total number of cells, but owing to the more

[12] *Loc. cit.*
[13] *Loc. cit.*

rapid division of the body cells this ratio decreases. In fact the chief difference between the yolk-free body cells and the (yolk-free) germ cells is the slow rate of division of the latter. Finally, the *eggs* will not divide at all unless specially stimulated, by fertilization. The question now arises: what causes the cell to divide. Sacks found that plant cells divide when they have reached a certain size. This rule has been extended to animals, and the final size of the cell found to be determined by the ratio of nucleus to cytoplasm. This rule may apply to the germ cells, since it appears that after the egg cell, primary oocyte, reaches a certain size any additional food absorbed does not cause growth of the protoplasm, but is precipitated as yolk.

If the egg is properly stimulated, rapid growth of protoplasm and cell divisions follow. From the study of artificial parthenogenesis it appears probable that stimuli which lead to development of the egg increases the permeability of its plasma membrane. If this be true we may say that the germ cells are distinguished by the fact that their plasma membranes are poorly permeable and retard those reactions between the cell contents and environment which lead to growth and cell division. In other words, the optimum intensity of stimulation toward growth and division is higher for the germ cells than for the body cells.

This difference is probably due to a difference in the colloids of the cell, which in animals could be explained as the result of an unequal mitosis. This explanation may be modified so as to apply to plants. Klebs has shown that those conditions which are adverse to vegetative growth of plants (too strong stimuli?) call forth flowers. Perhaps there are slight differences in the sensitiveness of plant cells to stimuli, and as the stimuli increase, those initially least sensitive cells acquire further immunity to the stimulus, whereas those initially more sensitive cells are overstimulated and weakened. Thus the difference between germ and body cells is grad-

ually acquired. The Malpighian layer of the skin may be stimulated to proliferate more rapidly, but if the stimuli are too strong the growth will be retarded instead of increased. On gradually increasing the stimulus immunity to it may be acquired.

To apply this hypothesis to the daphnids: conditions which are adverse to the growth of the body cells, such as extremes of temperature (viz., high temperatures) or of concentration of excretory products, or disorded nutrition, either fail to retard the development of the germ cells or stimulate their development, so that in either case the daphnid becomes sexually mature at a less developed stage. Under the less extreme conditions the eggs develop on receiving the slight stimulus incident to their transfer to the brood pouch, but under the more extreme conditions those eggs which develop at all must be stimulated by fertilization before they develop. These two types of eggs may perhaps develop from two kinds of cells, or the sexual egg may arise from the same kind of cell producing the parthenogenetic egg by acquiring an immunity to slight stimuli. Whereas more than one cell goes to make up a single egg; only one nucleus is retained, and it may be said that one cell is the egg cell and the remainder furnish its food.